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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12P 19/34, C07H 21/02, 21/04 | A1

(11) International Publication Number: WO 97/41263

(43) International Publication Date: 6 November 1997 (06.11.97)

(21) International Application Number: PCT/US97/07676

(22) International Filing Date: 28 April 1997 (28.04.97)

(30) Priority Data:
08/640,389 29 April 1996 (29.04.96) US

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(81) Designated States: AU, CA, IL, JP, KR, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: DETECTION OF THE LEPTIN RECEPTOR IN REPRODUCTIVE ORGANS AND METHODS FOR REGULATING REPRODUCTIVE BIOLOGY

(57) Abstract

The present invention relates to variant forms of the receptor for the *obese* gene product. In particular, the invention relates to methods of detecting receptor variants in the reproductive organs for the diagnosis of the cause of infertility. In addition, it relates to methods of inhibiting or down-regulating expression of defective variants in cells to augment their responsiveness to regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system to improve fertility.

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DETECTION OF THE LEPTIN RECEPTOR IN REPRODUCTIVE ORGANS AND METHODS FOR REGULATING REPRODUCTIVE BIOLOGY

1. INTRODUCTION

The present invention relates to variant forms of the receptor for the *obese* gene product. In particular, the invention relates to methods of detecting receptor variants in the reproductive organs for the diagnosis of the cause of infertility. In addition, it relates to methods of inhibiting or down-regulating expression of defective variants in cells to augment their responsiveness to regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system to improve fertility.

2. BACKGROUND OF THE INVENTION

Infertility is a major clinical problem in Western societies. A number of contributing factors have been identified for infertility, which include metabolic diseases in a male that result in insufficient sperm production and the inability of a female's ovaries to produce or release ova. In addition, pituitary disorders may cause infertility in both sexes because the gonads are responsive to regulation by pituitary hormones such as follicle-stimulating hormone and luteinizing hormone. However, clinical conditions exist in which an infertile female is still capable of ovulating. Therefore, the ovary may respond to additional signals that are not yet identified.

Zhang et al. (1994, *Nature* 372:425-432) describe the cloning and sequencing of the mouse *ob* gene and its human homolog. In an effort to understand the physiologic function of the *ob* gene, several independent research groups produced recombinant *ob* gene product in bacteria for *in vivo* testing (Pelleymounter et al., 1995, *Science* 269:540-543; Halaas et al., 1995, *Science* 269:543-546; Campfield et al., 1995, *Science* 269:546-549). When the Ob protein (also known as leptin) was injected into grossly obese mice, which possessed

two mutant copies of the *ob* gene, the mice exhibited a reduced appetite and began to lose weight. Similarly, when normal mice received leptin, they also ate less than the untreated controls. Interestingly, when leptin was administered to *ob/ob* female mice which were always infertile, fertility was restored in these animals (Chehab et al., 1996, *Nature Genetics* 12:318-320).

Recently, a leptin fusion protein was generated and used to screen for the leptin receptor (also known as OB-R) in a cDNA expression library prepared from mouse choroid plexus, a tissue that lines brain cavities termed ventricles (Tartaglia, 1995, *Cell* 83:1263-1271). This approach led to the cloning of one form of the OB-R coding sequence, which reveals a single membrane-spanning receptor, sharing structural similarities with several Class I cytokine receptors, such as the gp130 signal-transducing component of the interleukin-6 receptor (Taga et al., 1989, *Cell* 58:573-581), the granulocyte-colony stimulating factor receptor (Fukunaga et al., 1990, *Cell* 61:341-350), and the leukemia inhibitory factor receptor (Gearing et al., 1991, *EMBO J.* 10:2839-2848). Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) demonstrate that OB-R mRNA is expressed in several tissues, including lung, kidney, total brain, choroid plexus and hypothalamus.

The reported mouse OB-R protein contains a relatively short intracellular cytoplasmic domain as compared with other Class I cytokine receptors. Subsequently, when cDNA encoding its human homolog was isolated from a human infant brain library, the predicted human protein sequence contains a much longer intracellular domain. In view of this finding, it was speculated that different forms of the receptor might exist (Barinaga, 1996, *Science* 271:29). However, prior to the present invention, there was no report on how variant forms of the OB-R in humans would relate to infertility.

3. SUMMARY OF THE INVENTION

The present invention relates to variant forms of the human OB-R. In particular, it relates to the detection of these receptor variants in reproductive organs such as the ovary and the prostate gland for diagnosis of the cause of infertility, and methods for treating infertility by targeting these variant receptors.

The invention is based, in part, upon the Applicants' discovery of human cDNA clones encoding three variant forms of the OB-R. These receptors differ structurally from a reported OB-R with only three amino acid substitutions in the extracellular domain, but extensive diversity is observed in their intracellular cytoplasmic domains at the 3' end. The cytoplasmic domains of the variants of the invention are both shorter and distinct in nucleotide sequence from the corresponding domain of the published form of OB-R (Tartaglia et al., 1995 *Cell* 83:1263). In addition, the cytoplasmic domain of one such variant is highly homologous to a human retrotransposon sequence. The OB-R variants described herein represent incomplete receptors which may be incompetent or partially competent in transducing signals upon ligand binding. Expression of the different forms of the receptor have been detected in prostate and ovary. Furthermore, leptin activity has been shown to be naturally present in ovarian follicular fluids. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the detection of the receptor variants in reproductive organs for the diagnosis of infertility, methods to inhibit and/or down-regulate the expression of these receptor variants, gene therapy to replace the receptor variants in homozygous individuals, and direct activation of downstream signal transduction pathways in cells expressing the defective receptor variants for improving fertility.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1E. Nucleotide sequence and deduced amino acid sequence of Form 1 the human OB-R variant.

The amino acid sequence diverges from the human OB-R reported by Tartaglia et al. (1995, *Cell* 83:1263-1271) at nucleotide residue #349, #422, #764 and from residue #2770 and beyond.

Figure 2. Nucleotide sequence comparison between human OB-R variant Form 1, Form 2 and Form 3 at the 3' end.

Figure 3A-3C. Amino acid sequence comparison between OB-R variant Forms 1 (HuB1.219-1), 2 (HuB1.219-2), 3(HuB1.219-3), human OB-R (HuOBR) published by Tartaglia et al., 1995, *Cell* 83:1263; and murine OB-R (MuOBR).

Figure 4. Proliferation of BaF3 cells and cells transfected with chimeric OB-R in the presence of follicular fluids. ■ = transfected cell line; ⧈ = BaF3 parent cell line.

Figure 5. Proliferation of transfected BaF3 cell line in the presence of follicular fluids is inhibited by soluble OB-R, indicating that leptin is the active growth-inducing substance in the fluids. ■ = transfected cell line; ⧈ = BaF3 parent cell line.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE OB-R VARIANTS

The present invention relates to nucleic acid and amino acid sequences of OB-R variants. In a specific embodiment by way of example in Section 6, *infra*, three variants were cloned and characterized. Amino acid sequence comparison of these OB-R variants with a published human OB-R sequence (Tartaglia et al., 1995, *Cell* 83:1263-1271) reveals three amino acid differences in their extracellular domains and extensive diversity in their intracellular cytoplasmic

domains. More specifically, Figure 1A-1E shows that in the variants, nucleotide residues #349-351 encode alanine, nucleotide residues #421-423 encode arginine and nucleotide residues #763-765 encode arginine. Additionally, the variants diverge both in length and sequence composition from the human OB-R sequence published by Tartaglia et al. from nucleotide residue #2770 and beyond (Figure 2). In this regard, the intracellular domain of Form 1 (Figure 1A-1E) of the variants is highly homologous to a retrotransposon sequence (Ono *et al.*, 1987, *Nucl. Acid. Res.* 15:8725-8737). Such variants represent functionally defective forms of human OB-R in signal transduction upon leptin binding.

In order to clone additional variant forms of the molecule, labeled DNA probes made from nucleic acid fragments corresponding to any portion of the cDNA disclosed herein may be used to screen a cDNA library prepared from human ovary, human prostate, human fetal liver, human lung, human kidney, human choroid plexus and human hypothalamus. More specifically, oligonucleotides corresponding to either the 5' or 3' terminus of the cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon sperm DNA at 0.5 mg/ml for 6 hours at 60°C. The radiolabelled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution

containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M EDTA) twice for 5 minutes each at room temperature, then in 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is aligned with the filters to select a positive plaque. If a single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris Hcl, pH 7.5, 0.01% gelatin. The phage may then be replated and rescreened to obtain single, well isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

One method for identifying all 3' isoforms is to PCR amplify the 3' ends of the variant cDNA from a variety of tissues including but not limiting to, choroid plexus, hypothalamus, fetal liver, bone marrow, ovary, or prostate. To obtain the 3' end of the cDNA, an oligo-dT primer is used to synthesize the cDNA first strand. OB-R specific primers from the conserved region of the gene (e.g. up stream of nucleotide 2770) and oligo-dT are then used to amplify the 3' end. The PCR fragments are cloned and sequenced by standard techniques. Once obtained, these sequences may be translated into amino acid sequence and examined for certain landmarks such as continuous open reading frame, regulatory regions that associate with tyrosine kinase activation, and finally overall structural similarity to known OB-R variants. These 3' variants may represent additional signal transduction defective forms of OB-R.

5.2. EXPRESSION OF THE OB-R VARIANTS

In accordance with the invention, the OB-R variant polynucleotide sequence which encodes a protein, peptide fragments, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of the protein, peptide fragments, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a part of such polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used in the practice of the invention for the expression of the OB-R variant. Such DNA sequences include those which are capable of hybridizing to the OB-R variant sequence under stringent conditions, particularly at its 3' end. The phrase "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at PH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions,

additions or substitutions of amino acid residues within the OB-R variant sequence, which result in a silent change thus producing a functionally equivalent protein. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

The DNA sequence of the invention may be engineered in order to alter the OB-R variant coding sequence for a variety of ends, including but not limited to, alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, *e.g.*, site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. In addition, the intracellular domain may also be altered and replaced by a different domain, such as the OB-R intracellular domain by Tartaglia et al.

In another embodiment of the invention, the OB-R variant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or stimulators of receptor activity, it may be useful to encode a chimeric protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the OB-R variant sequence and the heterologous protein sequence, so that the variant may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of the OB-R variant could be synthesized in whole or

in part, using chemical methods well known in the art. (See, for example, Caruthers et al., 1980, *Nuc. Acids Res. Symp. Ser.* 7:215-233; Crea and Horn, 180, *Nuc. Acids Res.* *9(10)*:2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters 21*:719; and Chow and Kempe, 1981, *Nuc. Acids Res.* *9(12)*:2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize OB-R variant amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (*e.g.*, see Creighton, 1983, *Proteins Structures And Molecular Principles*, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

In order to express the OB-R variant in host cells, the nucleotide sequence coding for the variant, or a functional equivalent, is inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. The expressed gene products as well as host cells or cell lines transfected or transformed with recombinant OB-R variant expression vectors can be used for a variety of purposes. For example, host cells expressing the OB-R variant may be used to verify the ability of this molecule to bind leptin in a binding assay with radiolabeled, enzyme-conjugated or fluorescent dye-conjugated leptin. At the same time, the ability of the molecule to transduce an activation signal in host cells upon binding to leptin may be tested by assaying proliferation or phosphorylation pattern of kinases in the cells. In addition, genetically-engineered host cells can be used to screen for and select agonist and antagonist compounds, including any inhibitors that would interfere with binding of leptin to the extracellular or intracellular domain of the OB-R variant. In that connection, such host

cells may be used to screen for and select small molecules i.e., peptides, nucleic acids and synthetic compounds that can supplement the incomplete signal transduced by the OB-R variant following leptin binding. Such small molecules may also affect receptor isoform pairing, thereby modifying the ability of OB-R to respond to leptin. Other uses, include, but are not limited to generating antibodies (*i.e.*, monoclonal or polyclonal) that competitively inhibit activity of an OB-R variant, neutralize its activity, or even enhances it activity. Antibodies may be used in detecting and quantifying expression of OB-R levels in cells and tissues.

5.3. USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, an OB-R variant polynucleotide may be used to detect gene expression or aberrant gene expression in infertile individuals as well as in normal individuals to identify predisposition for infertility. Included in the scope of the invention are oligonucleotide sequences, that include antisense RNA and DNA molecules, ribozymes and triplex DNA, that function to inhibit translation of OB-R variant.

5.3.1. DIAGNOSTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may have a number of uses for the diagnosis of the possible causes underlying infertility, resulting from expression of a defective receptor variant. For example, the OB-R variant cytoplasmic domain DNA sequence may be used in hybridization assays of biopsy or autopsy materials obtained from ovary or prostate to diagnose OB-R variant expression; *e.g.*, Southern or Northern analysis, including *in situ* hybridization assays as well as PCR. Such techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits. For PCR detection, primers may be designed from a conserved region of the coding sequence and within the

3' region of OB-R variant. The tissues suitable for such analysis include but are not limited to, prostate, ovary, and testes, ova, sperm (semen), and cells in the ovarian follicular fluids.

5.3.2. THERAPEUTIC USES OF OB-R VARIANT POLYNUCLEOTIDES

An OB-R variant polynucleotide may be useful in the treatment of infertile conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not respond to leptin normally due to expression of a defective OB-R variant. In some instances, the polynucleotide encoding a functional OB-R is intended to replace or act in the place of the functionally defective OB-R variant gene. Alternatively, abnormal conditions characterized by expression of two copies of the OB-R variant can be treated using the gene therapy techniques described below.

Non-responsiveness to normal levels of leptin may contribute to infertility. This may result from a functionally defective receptor that does not transduce competent signals upon ligand binding. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express signalling competent forms of OB-R which may be used to augment the non-responsiveness of the naturally occurring OB-R variant. A signalling competent form may be, for example, a protein with the same extracellular domain and transmembrane region, but containing all or part of its normal signal transduction domain, such as that described by Tartaglia et al. (1995, *Cell* 83:1263-1271). Thus, recombinant gene therapy vectors may be used therapeutically for treatment of infertility resulting from expression or activity of the OB-R variant. Accordingly, the invention provides a method of augmenting signal transduction by an endogenous OB-R variant in a cell comprising delivering a DNA molecule encoding a signalling competent form of the OB-R to the cell so that the signalling competent protein is produced

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in the cell and competes with the endogenous defective OB-R variant for access to molecules in the signalling pathway which does not activate or are not activated by the endogenous natural defective receptor. Additionally, since dimerization of a functional receptor with a defective variant may occur in cells of heterozygous individuals, small molecules may be used to inhibit such pairing, thereby increasing the number of functional dimeric receptors for proper signalling in response to leptin.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant functional OB-R into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing an OB-R polynucleotide sequence. See, for example, the techniques described in Sambrook *et al.*, 1989, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. and Ausubel *et al.*, 1989, *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant OB-R molecules can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences including anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of the OB-R variant mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the OB-R variant nucleotide sequence at nucleotide #2771 and beyond, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the

invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of OB-R variant RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Oligodeoxyribonucleotides can form sequence-specific triple helices by hydrogen bonding to specific complementary sequences in duplexed DNA. Interest in triple helices has focused on the potential biological and therapeutic applications of these structures. Formation of specific triple helices may selectively inhibit the replication and/or gene expression of targeted genes by prohibiting the specific binding of functional trans-acting factors.

Oligonucleotides to be used in triplex helix formation should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Oligonucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich oligonucleotides provide base complementarily to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, oligonucleotides may be chosen that are purine-rich, for example, containing a stretch of G residues. These

oligonucleotides will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" oligonucleotide. Switchback oligonucleotides are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

5.4. ACTIVATION OF TYROSINE KINASE PATHWAYS IN INFERTILITY

Many known class I cytokine receptors initiate cell signaling via Janus kinases (JAKs) (Ihle, 1995, *Nature* 377:591-594; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al, 1994, *Cell* 76:253-62; Ziemiecki et al, 1994, *Trends Cell. Biol.* 4:207-212). JAK1-3 have been shown to bind to conserved sequences termed box1 and box2 (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53). Ligand binding induces a homo- or hetero-dimerization of receptor chains which activates, by phosphorylation, the JAKs. The activated JAKs, in turn, phosphorylate members of the STAT family (Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., *Blood* 86:1243-54; Darnell et al., 1994, *Science* 264:1415-21; Zhong et al, 1994, *Proc. Natl. Acad. Sci. USA* 91:4806-10; Hou et al., 1994, *Science* 265:1701-6). These phosphorylated STATs ultimately translocate to the nucleus, form transcription complexes, and regulate gene expression. Both box1 and box2 are required for complete signaling in certain systems. (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53).

The OB-R variants disclosed herein have a typical box1 (from nucleotide #2707-2730) that contains the critical xWxxxPxP amino acid sequence, but they do not contain an obvious box2 nor further downstream sequences that are important for normal receptor activation. Therefore, it is possible to use compounds that activate JAKs to directly activate these pathways for improving fertility without triggering the OB-R.

6. EXAMPLE: MOLECULAR CLONING OF OB-R VARIANTS

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (designated as Hu-B1.219 #4, #33, #34, #1, #3, #8, #36, #55, #57, #60 and #62) contained overlapping sequences,

which were then compiled into a contiguous nucleotide sequence referred to as Hu-B1.219. When the deduced amino acid sequence of one such sequence (Figure 1A-1E) was compared with the sequence of a recently published human OB-R (Tartaglia *et al.*, 1995, *Cell* 83:1263), they were shown to be nearly identical in the extracellular domains with the exception of three amino acids, whereas there existed extensive diversity in their intracellular cytoplasmic domains at the 3' end. This sequence encodes an OB-R variant herein referred to as Form 1. In addition, two other variants were identified, and they both differ from the human OB-R published by Tartaglia *et al* (1995, *Cell* 83:1263) in their 3' ends (Figure 2). These two additional variants are referred to as Forms 2 and 3 of OB-R. The predicted protein sequences of the variants (Figure 3A-3C) contain two FN III domains, each containing a "WS box", which are characteristic of genes of the Class I cytokine receptor family.

When various human tissue RNA were probed with a fragment containing a sequence commonly shared by the OB-R variants by Northern blot analysis, expression was detected in heart, placenta, lung, liver, muscle, pancreas, prostate, ovary, small intestine and brain (Table I).

Based on the sequence presented in Figure 1A-1E, the translation initiation site appears at position #97. The sequences of Forms 1, 2 and 3 encode an open reading frame up to and including nucleotide #2970, #2814 and #2784 (Figure 2), respectively. It is believed that the sequence between nucleotides #2629 and #2682 (Figure 1A-1E) encodes a transmembrane domain. The complete sequences of Forms 1, 2 and 3 encode proteins of 958, 906 and 896 amino acids, respectively.

TABLE I

SUMMARY OF NORTHERN BLOT ANALYSIS OF OB-R EXPRESSION IN HUMAN TISSUES AND CELL LINES

Developmental Stage	Tissue Type	Expression
fetal	brain	-
	lung	+++
	liver	+++++
	kidney	+
adult	heart	++
	brain	+/-
	placenta	+
	lung	+
	liver	+++
	skeletal muscle	+
	kidney	+/-
	pancreas	+
	spleen	+/-
	thymus	+/-
	prostate	++
	testis	+/-
	ovary	+++
	small intestine	++
	colon	-
	peripheral blood leukocytes	-

Developmental Stage	Tissue Type	Expression
cancer	HL-60	-
	HeLa	-
	K-562	+++
	MOLT-4	-
	Raji	-
	SW480	-
	A549	+
	G361	-

The sequences of the three OB-R variants are identical to the sequence of human OB-R reported by Tartaglia (1995, *Cell* 83:1263-1271) in the transmembrane region and a portion of the intracellular domain up to and including nucleotide #2769 (Figure 2), then they diverge at nucleotide #2770 and beyond. In addition, the products of these cDNA are substantially shorter in their intracellular domain than the human OB-R published by Tartaglia et al. These isoforms of OB-R may derive from a common precursor mRNA by an alternative splicing mechanism. The sequence in this region is consistent with well known splice junctions. It is noteworthy that the DNA sequence of Form 1 (Figure 1A-1E) of the OB-R variant from nucleotide #2768 to the end is 98% identical to a human retrotransposon sequence that is thought to be derived from a human endogenous retroviral DNA sequence (Singer, 1982, *Cell* 28:433; Weiner et al., 1986, *Ann. Rev. Biochem.* 55:631; Lower et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4480; Ono *et al.*, 1987, *Nucl. Acid. Res.* 15:8725-8735).

7. EXAMPLE: EXPRESSION OF OB-R IN SEX HORMONE PRODUCING CELLS IN THE OVARY AND DETECTION OF LEPTIN IN OVARIAN FOLLICULAR FLUIDS

7.1. MATERIALS AND METHODS

7.1.1. REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION (RT/PCR)

Total RNA was isolated using standard laboratory procedures (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Approximately 1 μg of total RNA was reverse transcribed and the cDNA was amplified by PCR (Perkin Elmer, Norwalk, CT). The PCR amplification conditions were: 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec for a total of 40 cycles. The amplified products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The OB-R amplimers were GGTTTGCATATGGAAGTC (upper) and CCTGAACCATCCAGTCTCT (lower). The Form 1 specific amplimers were GACTCATTGTGCAGTGTTCAG (upper) and TAGTGGAGGGAGGGTCAGCAG (lower).

7.2. RESULTS

Table I in Section 6, *supra*, shows that the OB-R is expressed in reproductive organs such as ovary and prostate gland. In order to determine the specific cell types in the ovary that expressed the receptor, primary granulosa and cumulus cell cultures were established from the ovaries and assayed for OB-R expression by RT/PCR. In Table II, the cells that produced sex hormones in the ovary, i.e. granulosa and cumulus cells, expressed the different forms of OB-R.

TABLE II

OB-R EXPRESSION IN GRANULOSA AND CUMULUS CELLS BY RT/PCR

Cell Types	Form 1	Form 3	OB-R*
Granulosa cells derived from ovarian follicles	+	+	+/-
Granulosa cells derived from ovarian follicles	-	+	+
Cumulus cells derived from oocytes	+	+	+

* OB-R refers to the published sequence by Tartaglia (1995, *Cell* 83:1263-1271).

Additionally, ovarian follicular fluids were obtained from several patients and assayed for the presence of leptin. The detection assay utilized an interleukin-3-dependent cell line, BaF3, that had been transfected with a chimeric receptor construct containing the extracellular domain of murine OB-R ligated to the transmembrane and cytoplasmic domains of the thrombopoietin receptor. Both the BaF3 parental cell line and the transfected cell line responded to IL-3, whereas only the transfected cells responded to leptin (Figure 4).

When the cells were incubated with follicular fluids, the transfected cell line was induced to proliferate as compared with the parental cell line as a control (Figure 4). The cell growth-stimulating activity in the fluids was shown to be leptin since the activity was specifically inhibited by the addition of soluble murine OB-R (Figure 5). Therefore, leptin is present in the follicular fluids, and it stimulates OB-R-expressing cells in the ovary to proliferate.

8. DEPOSIT OF MICROORGANISMS

The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

Strain Designation	Accession No.
HuB1.219, #1	75885
HuB1.219, #4	75886
HuB1.219, #8	75887
HuB1.219, #33	75888
HuB1.219, #34	75889
HuB1.219, #36	75890
HuB1.219, #55	75971
HuB1.219, #60	75973
HuB1.219, #3	75970
HuB1.219, #57	75972
HuB1.219, #62	75974

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of individual aspects of the invention. Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

International Application No: PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 36, lines 25-37 of the description

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit ' September 14, 1994 Accession Number ' 75885

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., 'Accession Number of Deposit')

E. ☐ This sheet was received with the International application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau '

was

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Form PCT/RO/134 (January 1981)

International Application No: PCT/

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

Accession No.	Date of Deposit
75886	September 14, 1994
75887	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75890	September 14, 1994
75970	December 14, 1994
75971	December 14, 1994
75972	December 14, 1994
75973	December 14, 1994
75974	December 14, 1994

WHAT IS CLAIMED IS:

1. A method for detecting a defective OB-R in cells comprising:

(a) extracting RNA from a cell population;

(b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 1 in Figure 2; and

(c) detecting hybridization of the RNA with the oligonucleotide.

2. The method of Claim 1 in which the cell population is obtained from ovary.

3. The method of Claim 1 in which the cell population is obtained from prostate.

4. The method of Claim 1 in which the cell population is obtained from testis.

5. The method of Claim 1 in which the cell population is obtained from sperm.

6. The method of Claim 1 in which the cell population is obtained from ovum.

7. The method of Claim 1 in which the cell population is obtained from cells of ovarian follicular fluids.

8. The method of Claim 1 in which the cell population is obtained from blood.

9. A method for detecting a defective OB-R in cells comprising:

(a) extracting RNA from a cell population;

(b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 2 in Figure 2; and

(c) detecting hybridization of the RNA with the oligonucleotide.

10. The method of Claim 9 in which the cell population is obtained from ovary.

11. The method of Claim 9 in which the cell population is obtained from prostate.

12. The method of Claim 9 in which the cell population is obtained from testis.

13. The method of Claim 9 in which the cell population is obtained from sperm.

14. The method of Claim 9 in which the cell population is obtained from ovum.

15. The method of Claim 9 in which the cell population is obtained from cells of ovarian follicular fluids.

16. The method of Claim 9 in which the cell population is obtained from blood.

17. A method for detecting a defective OB-R in cells comprising:

(a) extracting RNA from a cell population;
(b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted as Form 3 in Figure 2; and
(c) detecting hybridization of the RNA with the oligonucleotide.

18. The method of Claim 17 in which the cell population is obtained from ovary.

19. The method of Claim 17 in which the cell population is obtained from prostate.

20. The method of Claim 17 in which the cell population is obtained from testis.

21. The method of Claim 17 in which the cell population is obtained from sperm.

22. The method of Claim 17 in which the cell population is obtained from ovum.

23. The method of Claim 17 in which the cell population is obtained from cells of ovarian follicular fluids.

24. The method of Claim 17 in which the cell population is obtained from blood.

25. A method for treating infertility, comprising administering to an individual an effective amount of an agent capable of inhibiting expression of an OB-R variant gene.

26. The method of Claim 25 in which the OB-R variant gene further comprises the sequence of Figure 2 or which is capable of selectively hybridizing to it.

27. The method of Claim 26 in which the agent is an antisense molecule complementary to mRNA encoded by the sequence of Figure 2.

28. The method of Claim 26 in which the agent is a ribozyme molecule specific for mRNA encloded by the sequence of Figure 2.

29. The method of Claim 26 in which the agent is a triple helix component.

30. A method for identifying a compound capable of supplementing biological activity of leptin on cells of reproductive organs, comprising:

(a) incubating the cells expressing an OB-R variant with leptin;

(b) incubating a portion of the leptin-treated cells with a test compound; and

(c) comparing activation signal in the cells treated in step (b) with cells treated in step (a);

thereby determining the compound that augments activation of the OB-R variant by leptin.

31. The method of Claim 30 in which the OB-R variant is encoded by the sequence depicted in Figure 2.

32. A method for identifying a compound capable of inhibiting biological activity of leptin on cells of reproductive organs, comprising:

(a) incubating the cells expressing an OB-R variant with leptin;

(b) incubating a portion of the leptin-treated cells with a test compound; and

(c) comparing activation signal in the cells treated in step (b) with cells treated in step (a);

thereby determining the compound that inhibits activation of the OB-R variant by leptin.

33. The method of Claim 32 in which the OB-R variant is encoded by the sequence depicted in Figure 2.

```
                9                  18                 27                 36                 45                 54
GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC TTC GGT
 A   R   A   T   Q   V   P   E   P   R   P   A   P   I   S   A   F   G

               63                 72                 81                 90                 99                108
CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG ATG ATT TGT CAA
 R   V   G   P   P   D   Q   G   V   L   L   *   S   K   M   I   C   Q

              117                126                135                144                153                162
AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT TAT GTG ATA ACT GCG TTT
 K   F   C   V   V   L   L   H   W   E   F   I   Y   V   I   T   A   F

              171                180                189                198                207                216
AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA TTT AAG TTG TCT TGC ATG CCA CCA
 N   L   S   Y   P   I   T   P   W   R   F   K   L   S   C   M   P   P

              225                234                243                252                261                270
AAT TCA ACC TAT GAC TAC TTC CTT TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA
 N   S   T   Y   D   Y   F   L   L   P   A   G   L   S   K   N   T   S

              279                288                297                306                315                324
AAT TCG AAT GGA CAT TAT GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT
 N   S   N   G   H   Y   E   T   A   V   E   P   K   F   N   S   S   G

              333                342                351                360                369                378
ACT CAC TTT TCT AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CGG AGT GAG
 T   H   F   S   N   L   S   K   A   T   F   H   C   C   F   R   S   E

              387                396                405                414                423                432
CAA GAT AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT
 Q   D   R   N   C   S   L   C   A   D   N   I   E   G   R   T   F   V   =

              441                450                459                468                477                486
TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC ATA CAG
 S   T   V   N   S   L   V   F   Q   Q   I   D   A   N   W   N   I   Q

              495                504                513                522                531                540
TGC TGG CTA AAA GGA GAC TTA AAA TTA TTC ATC TGT TAT GTG GAG TCA TTA TTT
 C   W   L   K   G   D   L   K   L   F   I   C   Y   V   E   S   L   F

              549                558                567                576                585                594
AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT CTT TTA TAT GTT CTG CCT
 K   N   L   F   R   N   Y   N   Y   K   V   H   L   L   Y   V   L   P

              603                612                621                630                639                648
GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC CAA AAA GGC AGT TTT CAG ATG GTT
 E   V   L   E   D   S   P   L   V   P   Q   K   G   S   F   Q   M   V
```

Figure 1A

```
                657                 666                 675                 684                 693                 702
CAC TGC AAT TGC AGT GTT CAT GAA TGT TGT GAA TGT CTT GTG CCT GTG CCA ACA
---------------------------------------------------------------------
 H   C   N   C   S   V   H   E   C   C   E   C   L   V   P   V   P   T

                711                 720                 729                 738                 747                 756
GCC AAA CTC AAC GAC ACT CTC CTT ATG TGT TTG AAA ATC ACA TCT GGT GGA GTA
---------------------------------------------------------------------
 A   K   L   N   D   T   L   L   M   C   L   K   I   T   S   G   G   V

                765                 774                 783                 792                 801                 810
ATT TTC CGG TCA CCT CTA ATG TCA GTT CAG CCC ATA AAT ATG GTG AAG CCT GAT
---------------------------------------------------------------------
 I   F   R   S   P   L   M   S   V   Q   P   I   N   M   V   K   P   D

                819                 828                 837                 846                 855                 864
CCA CCA TTA GGT TTG CAT ATG GAA ATC ACA GAT GAT GGT AAT TTA AAG ATT TCT
---------------------------------------------------------------------
 P   P   L   G   L   H   M   E   I   T   D   D   G   N   L   K   I   S

                873                 882                 891                 900                 909                 918
TGG TCC AGC CCA CCA TTG GTA CCA TTT CCA CTT CAA TAT CAA GTG AAA TAT TCA
---------------------------------------------------------------------
 W   S   S   P   P   L   V   P   F   P   L   Q   Y   Q   V   K   Y   S

                927                 936                 945                 954                 963                 972
GAG AAT TCT ACA ACA GTT ATC AGA GAA GCT GAC AAG ATT GTC TCA GCT ACA TCC
---------------------------------------------------------------------
 E   N   S   T   T   V   I   R   E   A   D   K   I   V   S   A   T   S

                981                 990                 999                1008                1017                1026
CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT GAG GTT CAG GTG AGG GGC
---------------------------------------------------------------------
 L   L   V   D   S   I   L   P   G   S   S   Y   E   V   Q   V   R   G

               1035                1044                1053                1062                1071                1080
AAG AGA CTG GAT GGC CCA GGA ATC TGG AGT GAC TGG AGT ACT CCT CGT GTC TTT
---------------------------------------------------------------------
 K   R   L   D   G   P   G   I   W   S   D   W   S   T   P   R   V   F

               1089                1098                1107                1116                1125                1134
ACC ACA CAA GAT GTC ATA TAC TTT CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT =
---------------------------------------------------------------------
 T   T   Q   D   V   I   Y   F   P   P   K   I   L   T   S   V   G   S

               1143                1152                1161                1170                1179                1188
AAT GTT TCT TTT CAC TGC ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA
---------------------------------------------------------------------
 N   V   S   F   H   C   I   Y   K   K   E   N   K   I   V   P   S   K

               1197                1206                1215                1224                1233                1242
GAG ATT GTT TGG TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT
---------------------------------------------------------------------
 E   I   V   W   W   M   N   L   A   E   K   I   P   Q   S   Q   Y   D

               1251                1260                1269                1278                1287                1296
GTT GTG AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA
---------------------------------------------------------------------
 V   V   S   D   H   V   S   K   V   T   F   F   N   L   N   E   T   K

               1305                1314                1323                1332                1341                1350
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT GAA TGC
---------------------------------------------------------------------
 P   R   G   K   F   T   Y   D   A   V   Y   C   C   N   E   H   E   C
```

Figure 1B

```
                1359                1368                1377                1386                1395                1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT ATC TCA TGT
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 H   H   R   Y   A   E   L   Y   V   I   D   V   N   I   N   I   S   C

                1413                1422                1431                1440                1449                1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC AGT ACA ATC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 E   T   D   G   Y   L   T   K   M   T   C   R   W   S   T   S   T   I

                1467                1476                1485                1494                1503                1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC AGC CTT TAC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 Q   S   L   A   E   S   T   L   Q   L   R   Y   H   R   S   S   L   Y

                1521                1530                1539                1548                1557                1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 C   S   D   I   P   S   I   H   P   I   S   E   P   K   D   C   Y   L

                1575                1584                1593                1602                1611                1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 Q   S   D   G   F   Y   E   C   I   F   Q   P   I   F   L   L   S   G

                1629                1638                1647                1656                1665                1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 Y   T   M   W   I   R   I   N   H   S   L   G   S   L   D   S   P   P

                1683                1692                1701                1710                1719                1728
ACA TGT GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC AGT GTG AAA
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 T   C   V   L   P   D   S   V   V   K   P   L   P   P   S   S   V   K

                1737                1746                1755                1764                1773                1782
GCA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA AAG CCA GTC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 A   E   I   T   I   N   I   G   L   L   K   I   S   W   E   K   P   V

                1791                1800                1809                1818                1827                1836
TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA AGT GGA AAA GAA =
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 F   P   E   N   N   L   Q   F   Q   I   R   Y   G   L   S   G   K   E

                1845                1854                1863                1872                1881                1890
GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT GCA AAA TCA AAA TCT GTC AGT CTC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 V   Q   W   K   M   Y   E   V   Y   D   A   K   S   K   S   V   S   L

                1899                1908                1917                1926                1935                1944
CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT GTT CAG GTG CGC TGT AAG AGG CTA
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 P   V   P   D   L   C   A   V   Y   A   V   Q   V   R   C   K   R   L

                1953                1962                1971                1980                1989                1998
GAT GGA CTG GGA TAT TGG AGT AAT TGG AGC AAT CCA GCC TAC ACA GTT GTC ATG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 D   G   L   G   Y   W   S   N   W   S   N   P   A   Y   T   V   V   M

                2007                2016                2025                2034                2043                2052
GAT ATA AAA GTT CCT ATG AGA GGA CCT GAA TTT TGG AGA ATA ATT AAT GGA GAT
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 D   I   K   V   P   M   R   G   P   E   F   W   R   I   I   N   G   D
```

Figure 1C

```
          2061                2070                2079                2088                2097                2106
ACT ATG AAA AAG GAG AAA AAT GTC ACT TTA CTT TGG AAG CCC CTG ATG AAA AAT
 T   M   K   K   E   K   N   V   T   L   L   W   K   P   L   M   K   N

          2115                2124                2133                2142                2151                2160
GAC TCA TTG TGC AGT GTT CAG AGA TAT GTG ATA AAC CAT CAT ACT TCC TGC AAT
 D   S   L   C   S   V   Q   R   Y   V   I   N   H   H   T   S   C   N

          2169                2178                2187                2196                2205                2214
GGA ACA TGG TCA GAA GAT GTG GGA AAT CAC ACG AAA TTC ACT TTC CTG TGG ACA
 G   T   W   S   E   D   V   G   N   H   T   K   F   T   F   L   W   T

          2223                2232                2241                2250                2259                2268
GAG CAA GCA CAT ACT GTT ACG GTT CTG GCC ATC AAT TCA ATT GGT GCT TCT GTT
 E   Q   A   H   T   V   T   V   L   A   I   N   S   I   G   A   S   V

          2277                2286                2295                2304                2313                2322
GCA AAT TTT AAT TTA ACC TTT TCA TGG CCT ATG AGC AAA GTA AAT ATC GTG CAG
 A   N   F   N   L   T   F   S   W   P   M   S   K   V   N   I   V   Q

          2331                2340                2349                2358                2367                2376
TCA CTC AGT GCT TAT CCT TTA AAC AGC AGT TGT GTG ATT GTT TCC TGG ATA CTA
 S   L   S   A   Y   P   L   N   S   S   C   V   I   V   S   W   I   L

          2385                2394                2403                2412                2421                2430
TCA CCC AGT GAT TAC AAG CTA ATG TAT TTT ATT ATT GAG TGG AAA AAT CTT AAT
 S   P   S   D   Y   K   L   M   Y   F   I   I   E   W   K   N   L   N

          2439                2448                2457                2466                2475                2484
GAA GAT GGT GAA ATA AAA TGG CTT AGA ATC TCT TCA TCT GTT AAG AAG TAT TAT
 E   D   G   E   I   K   W   L   R   I   S   S   S   V   K   K   Y   Y

          2493                2502                2511                2520                2529                2538
ATC CAT GAT CAT TTT ATC CCC ATT GAG AAG TAC CAG TTC AGT CTT TAC CCA ATA =
 I   H   D   H   F   I   P   I   E   K   Y   Q   F   S   L   Y   P   I

          2547                2556                2565                2574                2583                2592
TTT ATG GAA GGA GTG GGA AAA CCA AAG ATA ATT AAT AGT TTC ACT CAA GAT GAT
 F   M   E   G   V   G   K   P   K   I   I   N   S   F   T   Q   D   D

          2601                2610                2619                2628                2637                2646
ATT GAA AAA CAC CAG AGT GAT GCA GGT TTA TAT GTA ATT GTG CCA GTA ATT ATT
 I   E   K   H   Q   S   D   A   G   L   Y   V   I   V   P   V   I   I

          2655                2664                2673                2682                2691                2700
TCC TCT TCC ATC TTA TTG CTT GGA ACA TTA TTA ATA TCA CAC CAA AGA ATG AAA
 S   S   S   I   L   L   L   G   T   L   L   I   S   H   Q   R   M   K

          2709                2718                2727                2736                2745                2754
AAG CTA TTT TGG GAA GAT GTT CCG AAC CCC AAG AAT TGT TCC TGG GCA CAA GGA
 K   L   F   W   E   D   V   P   N   P   K   N   C   S   W   A   Q   G
```

Figure 1D

```
                2763                2772                2781                2790                2799                2808
CTT AAT TTT CAG AAG ATG CTT GAA GGC AGC ATG TTC GTT AAG AGT CAT CAC CAC
L   N   F   Q   K   M   L   E   G   S   M   F   V   K   S   H   H   H

                2817                2826                2835                2844                2853                2862
TCC CTA ATC TCA AGT ACC CAG GGA CAC AAA CAC TGC GGA AGG CCA CAG GGT CCT
S   L   I   S   S   T   Q   G   H   K   H   C   G   R   P   Q   G   P

                2871                2880                2889                2898                2907                2916
CTG CAT AGG AAA ACC AGA GAC CTT TGT TCA CTT GTT TAT CTG CTG ACC CTC CCT
L   H   R   K   T   R   D   L   C   S   L   V   Y   L   L   T   L   P

                2925                2934                2943                2952                2961                2970
CCA CTA TTG TCC TAT GAC CCT GCC AAA TCC CCC TCT GTG AGA AAC ACC CAA GAA
P   L   L   S   Y   D   P   A   K   S   P   S   V   R   N   T   Q   E

                2979                2988
TGA TCA ATA AAA AAA AAA AAA 3'
*   S   I   K   K   K   K
```

Figure 1 E

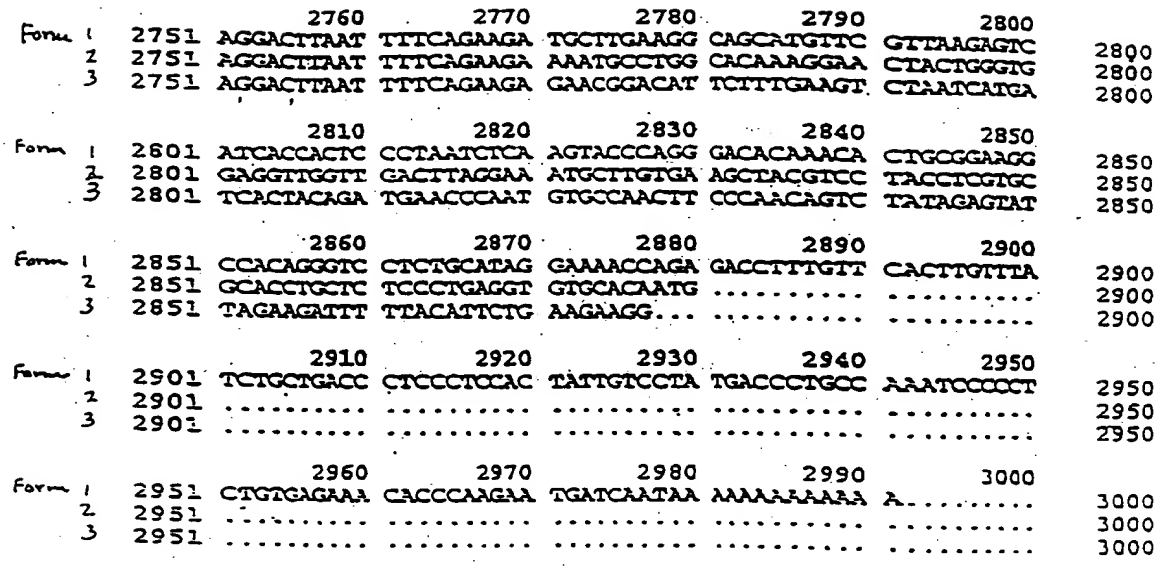
2760 2770 2780 2790 2800
Form 1 2751 AGGACTTAAT TTTCAGAAGA TGCTTGAAGG CAGCATGTTC GTTAAGAGTC 2800
2 2751 AGGACTTAAT TTTCAGAAGA AAATGCCTGG CACAAAGGAA CTACTGGGTG 2800
3 2751 AGGACTTAAT TTTCAGAAGA GAACGGACAT TCTTTGAAGT CTAATCATGA 2800
2810 2820 2830 2840 2850
Form 1 2601 ATCACCACTC CCTAATCTCA AGTACCCAGG GACACAAACA CTGCGGAAGG 2850
2 2801 GAGGTTGGTT GACTTAGGAA ATGCTTGTGA AGCTACGTCC TACCTCGTGC 2850
3 2801 TCACTACAGA TGAACCCAAT GTGCCAACTT CCCAACAGTC TATAGAGTAT 2850
2860 2870 2880 2890 2900
Form 1 2851 CCACAGGGTC CTCTGCATAG GAAAACCAGA GACCTTTGTT CACTTGTTTA 2900
2 2851 GCACCTGCTC TCCCTGAGGT GTGCACAATG 2900
3 2851 TAGAAGATTT TTACATTCTG AAGAAGG... 2900
2910 2920 2930 2940 2950
Form 1 2901 TCTGCTGACC CTCCCTCCAC TATTGTCCTA TGACCCTGCC AAATCCCCCT 2950
2 2901 2950
3 2901 2950
2960 2970 2980 2990 3000
Form 1 2951 CTGTGAGAAA CACCCAAGAA TGATCAATAA AAAAAAAAAA A......... 3000
2 2951 3000
3 2951 3000

Figure 2.

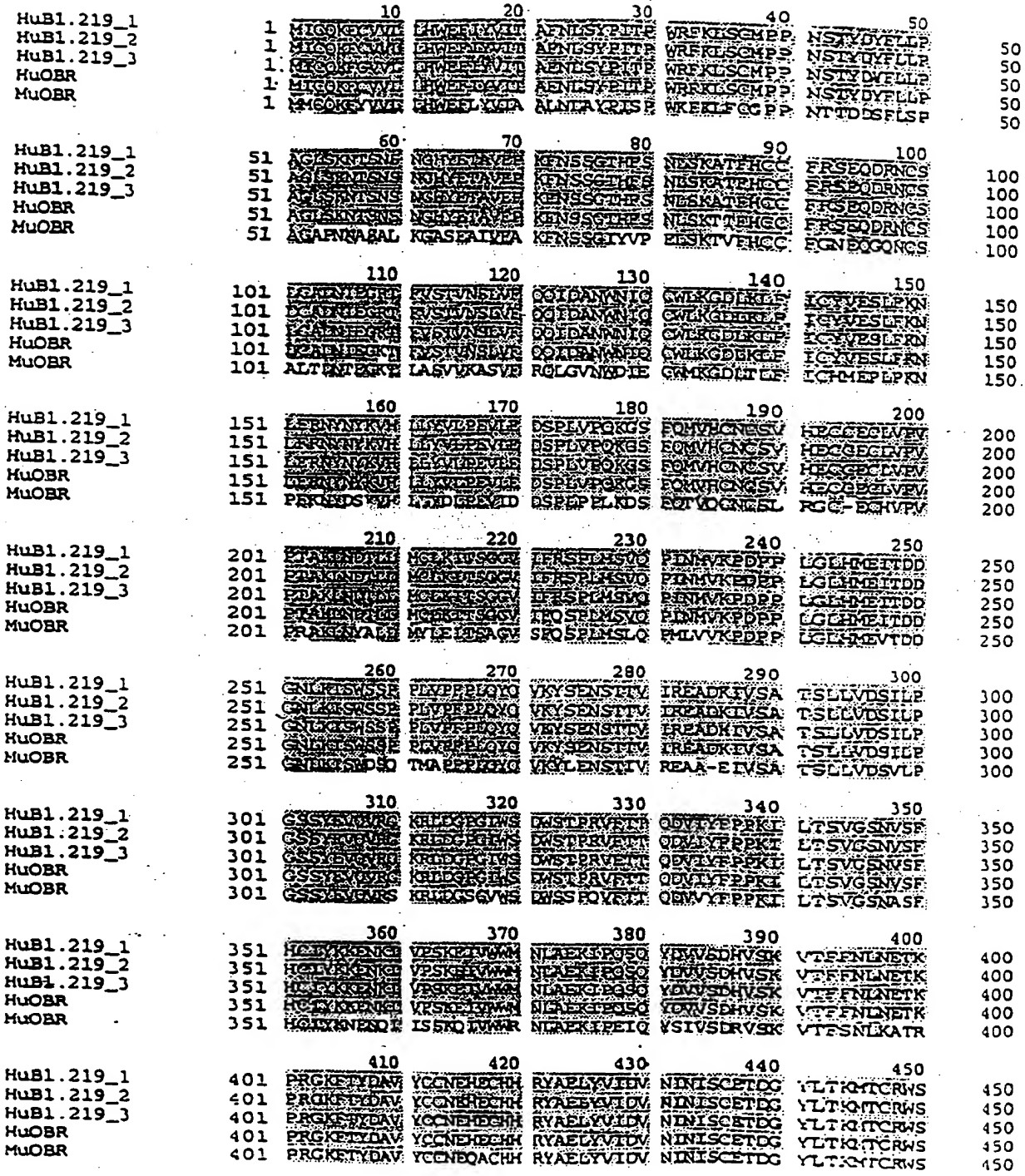

Figure 3A

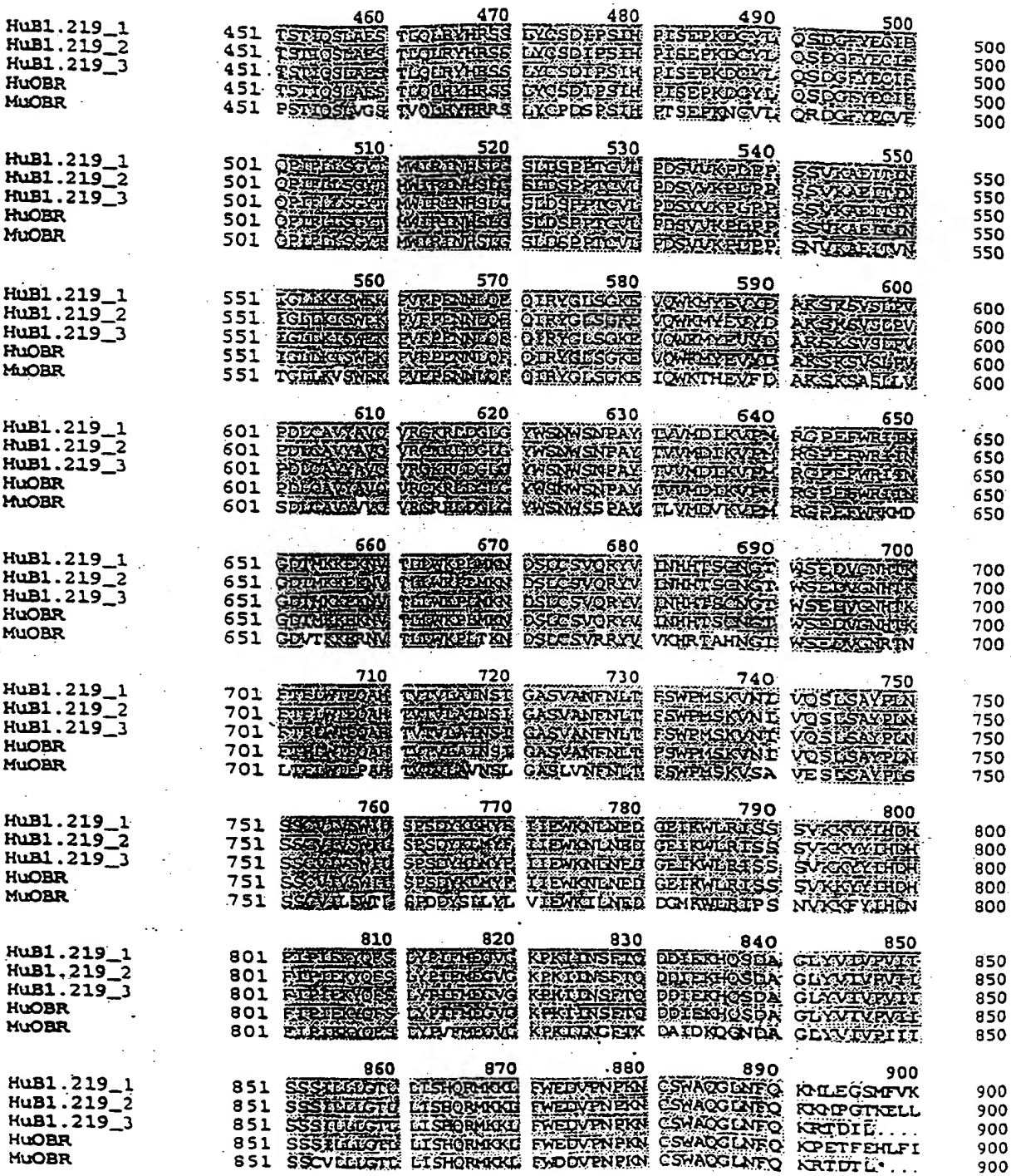

Figure 3B

```
                                  910        920        930        940        950
HuB1.219_1      901 SHHHSLISST QGHKHCGRPQ GPLHRKTRDL CSLVYLLTLP PEESYDPAKS 950
HuB1.219_2      901 GGGWLT.... .......... .......... .......... .......... 950
HuB1.219_3      901 .......... .......... .......... .......... .......... 950
HuOBR           901 KHTASVTCGP LLLEPETISE DISVDTSWKN KDEMMPTTVV SEESTTDLEK 950
MuOBR           901 .......... .......... .......... .......... .......... 950

                                  960        970        980        990       1000
HuB1.219_1      951 PSVRNTQE.. .......... .......... .......... .......... 1000
HuB1.219_2      951 .......... .......... .......... .......... .......... 1000
HuB1.219_3      951 .......... .......... .......... .......... .......... 1000
HuOBR           951 GSVCISDQFN SVNFSEAEGT EVTYEAESQR QPFVKYATLI SNSKPSETGE 1000
MuOBR           951 .......... .......... .......... .......... .......... 1000

                                 1010       1020       1030       1040       1050
HuB1.219_1     1001 .......... .......... .......... .......... .......... 1050
HuB1.219_2     1001 .......... .......... .......... .......... .......... 1050
HuB1.219_3     1001 .......... .......... .......... .......... .......... 1050
HuOBR          1001 EQGLINSSVT KCFSSKNSPL KDSFSNSSWE IEAQAFFILS DQHPNIISPH 1050
MuOBR          1001 .......... .......... .......... .......... .......... 1050

                                 1060       1070       1080       1090       1100
HuB1.219_1     1051 .......... .......... .......... .......... .......... 1100
HuB1.219_2     1051 .......... .......... .......... .......... .......... 1100
HuB1.219_3     1051 .......... .......... .......... .......... .......... 1100
HuOBR          1051 LTFSEGLDEL LKLEGNFPEE NNDKKSIYYL GVTSIKKRES GVLLTDKSRV 1100
MuOBR          1051 .......... .......... .......... .......... .......... 1100

                                 1110       1120       1130       1140       1150
HuB1.219_1     1101 .......... .......... .......... .......... .......... 1150
HuB1.219_2     1101 .......... .......... .......... .......... .......... 1150
HuB1.219_3     1101 .......... .......... .......... .......... .......... 1150
HuOBR          1101 SCPFPAPCLF TDIRVLQDSC SHFVENNINL GTSSKKTFAS YMPQFQTCST 1150
MuOBR          1101 .......... .......... .......... .......... .......... 1150

                                 1160       1170       1180       1190       1200
HuB1.219_1     1151 .......... .......... .......... .......... .......... 1200
HuB1.219_2     1151 .......... .......... .......... .......... .......... 1200
HuB1.219_3     1151 .......... .......... .......... .......... .......... 1200
HuOBR          1151 QTHKCMENKH CDLTV*.... .......... .......... .......... 1200
MuOBR          1151 .......... .......... .......... .......... .......... 1200
```

Figure 3C

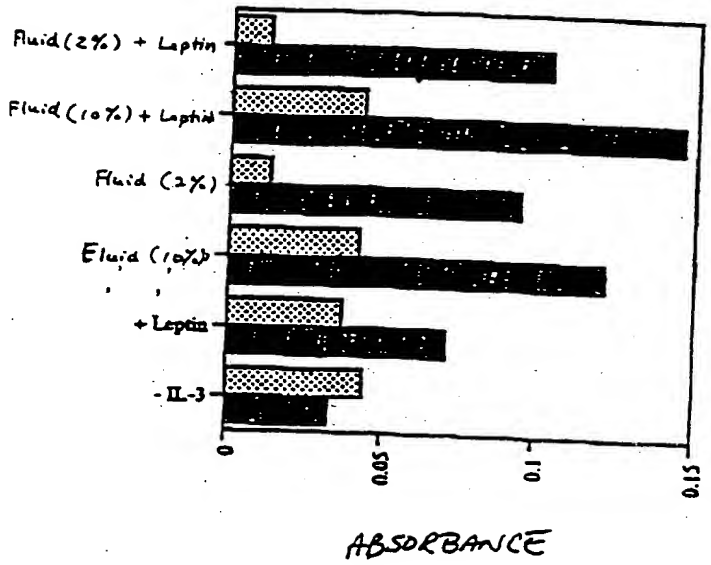
Fluid (2%) + Leptin
Fluid (10%) + Leptin
Fluid (2%)
Fluid (10%)
+ Leptin
- IL-3
0
0.05
0.1
0.15
ABSORBANCE

Figure 4

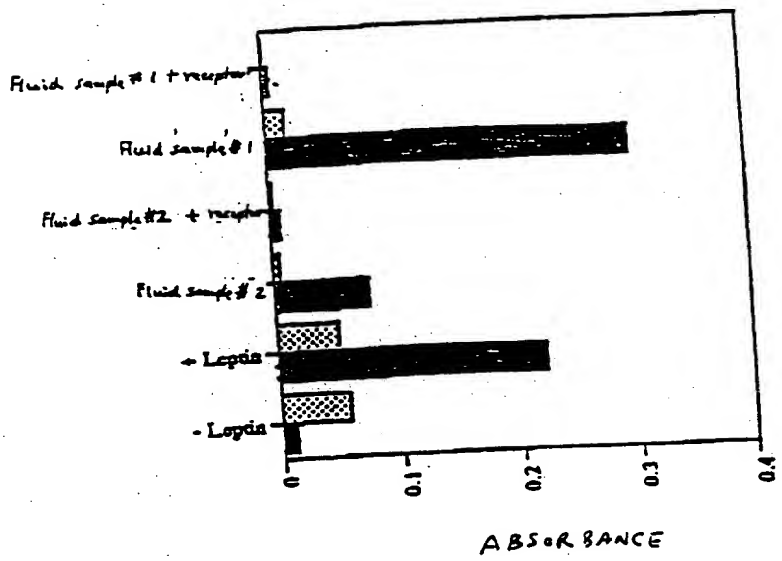
Fluid sample # 1 + receptor
Fluid sample # 1
Fluid sample #2 + receptor
Fluid sample # 2
+ Leptin
- Leptin
0
0.1
0.2
0.3
0.4
ABSORBANCE

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US97/07676

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68; C12P 19/34; C07H 21/02, 21/04
US CL :435/6, 91.2; 536/23.1, 24.3, 24.33
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.2; 536/23.1, 24.3, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	TARTAGLIA, L.A. et al., Identification and Expression Cloning of a Leptin Receptor, OB-R. Cell . 29 December 1995. Volume 83, pages 1263-1271, see entire document.	1,9,17, 25,26,30, 32 ---------- 2-8, 10-16, 18-24, 27-29, 31, 33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 JULY 1997	04.08.97
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DIANNE REES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07676

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used).

APS, BIOSIS, BIOTECHABS, BIOTECHDS, CABA,CAPLUS, CANCERLIT, EMBASE, MEDLINE, USPATFULL, EUROPATFULL, JAPIO,GENBANK, SCISEARCH, LIFESCI, TOXLINE,TOXLIT, DRUGU
search terms: leptin receptor, OB-R , probes, primers, sequence, cloning, diagnosis, mutation detection, alleles, variants, forms, isoforms,, ribozymes, antisense, triple helix, screening, drugs